TABLE 1
Inhibition of Appressorium Formation in *Magnaporthe grisea* by Glisoprenins A, C, D and E and Fatty Acids on a Hydrophobic (A) and Hydrophilic (B, C) Surface

Compound	AIC_{80}[a] (mg litre^{-1})		
	A[b]	B[c]	C[d]
Glisoprenin A or C or D	4	>100	>100
Glisoprenin E	40	>100	>100
Palmitoleic acid	10	10	>100
Petroselinic acid	10	20	>100
Petroselaidic acid	20	20	>100
Oleic acid	20	2	>100
Elaidic acid	20	1	>100
cis-Vaccenic acid	20	20	>100
trans-Vaccenic acid	>100	10	>100

[a] AIC_{80}: Concentration at which appressorium formation was inhibited by 80(±5)%.
[b] GelBond sheet, control: 95·6(±2·4)% of the germinating conidia formed appressoria.
[c] Induction with 0·2 mg litre^{-1} 1,16-hexadecanediol: 91·4(±3.6)% of the germinated conidia formed appressoria.
[d] Induction with 25 mg litre^{-1} chlorophenylthio-cAMP 94·6(±2·1)% of the germinated conidia formed appressoria.

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A New Type of Plant Activator: Thieno[2,3-*d*][1,2,3]thiadiazole-6-carboxylic Acid Derivatives

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Abstract: A short and efficient synthesis of a series of the title compounds is presented starting with methylenebutanedioic acid and thioacetic acid. Using the Hurd–Mori reaction in the key step, the optimised reaction sequence allows the large-scale preparation of this new type of plant activator in a few steps with a high overall yield. Additional functionalisation of the 5-position via directed *ortho*-lithiation methodology is also described. © 1998 Society of Chemical Industry

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Key words: plant activators; systemic acquired resistance; chemically induced resistance; thieno[2,3-*d*][1,2,3]thiadiazoles; Hurd–Mori reaction; directed *ortho*-lithiation

It has long been known that plants can develop a long-lasting broad-spectrum resistance against subsequent infections when locally infected with pathogens. In the course of extensive studies of this phenomenon it was discovered that induction of disease resistance in plants, called 'systemic acquired resistance' (SAR), can also be triggered by selected organic compounds which today are known as plant activators, examples being 2,6-

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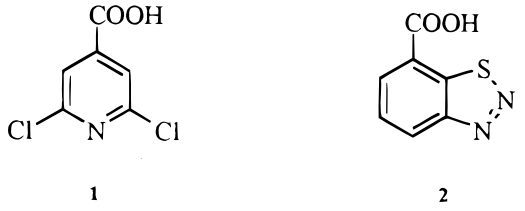

Fig. 1. Examples of plant activators.

dichloroisonicotinic acid (Fig. 1; **1**) and benzo[1,2,3]-thiadiazole-7-carboxylic acid (**2**). At concentrations which control disease *in vivo*, these compounds have little or no direct antimicrobial activity *in vitro* but they induce resistance in the plant to the same spectrum of pathogens as in nature and lead to the expression of the same biochemical markers, such as PR-proteins in the plant. For reviews of this phenomenon, see References 1–3. As a consequence, chemically mediated SAR was recently established as a new concept in disease control and the thiomethylester of **2** was introduced as the first commercial product (Bion®) by Novartis in 1996.[4]

In the course of our synthetic work towards derivatives of thieno[2,3-*d*][1,2,3]thiadiazole-6-carboxylic acid[5]—a new class of compounds which are bioisosteric to **2**—we applied the Hurd–Mori reaction[6] for the anellation of the 1,2,3-thiadiazole ring system as an alternative to the diazotisation approach.

Thus, 1,4-addition of thioacetic acid to methylenebutanedioic acid **3** yielded the addition product according to Holmberg,[7] and this intermediate was then hydrolysed to the thiol **4**, which was converted to the thiolactone **5** simply by heating to 140°C. To avoid cleavage of the sensitive thiolactone moiety, **5** was esterified with methanol and dicyclohexyl carbodiimide. Selective thionation to the corresponding dithiolactone **6** with Lawesson's reagent and subsequent condensation with ethylcarbazate in refluxing ethanol led to the pure *Z*-isomer of the carbazonate **7**, as established by X-ray analysis.

Our first attempt at the anellation of the 1,2,3-thiadiazole ring system involved treatment of **7** at room temperature with thionyl chloride (10 equiv.) in dichloromethane. After 22 h, a mixture of the final target compound **8** and a by-product, identified as the chlorinated thienothiadiazole **9**, was isolated (ratio: 8 : 1) instead of the expected 5,6-dihydro-thienothiadiazole (Fig. 2). Further work on the optimisation of reaction conditions showed that the formation of **9** could be avoided simply by raising the reaction temperature to 80°C.[8]

The unexpected aromatisation in the course of the cyclisation reaction, as well as the formation of the chlorinated by-product **9**, can be explained by a modified mechanistic model of the Hurd–Mori reaction which is depicted in Fig. 3. The first step is the cyclisation of **7** with thionyl chloride to the *N*-ethoxycarbonyl-dihydrothiadiazole *S*-oxide 10.[9] A Pummerer-like rearrangement initiated by the attack of thionyl chloride on the O-atom of the SO-group, followed by subsequent elimination of hydrochloric acid, led to the intermediate **11**. Addition of thionyl chloride to the push–pull substituted double bond furnished the sulfinylchloride **12** which formed **13** by *syn*-elimination.[10] The *N*-ethoxycarbonyl-thiadiazolium chloride **13** could now lose ethyl chloroformate to give **8** as the main product, or its push–pull substituted double bond could again add thionyl chloride to yield the corresponding sulfinyl chloride **14**, which gave the by-product **9**, again via *syn*-elimination and subsequent aromatisation. At higher reaction temperatures the aromatisation of **13** is obviously preferred to the addition of thionyl chloride, leading exclusively to **8** (see also Fig. 2).

Due to the interesting biological profile of the by-product **9** we were interested in an efficient synthetic

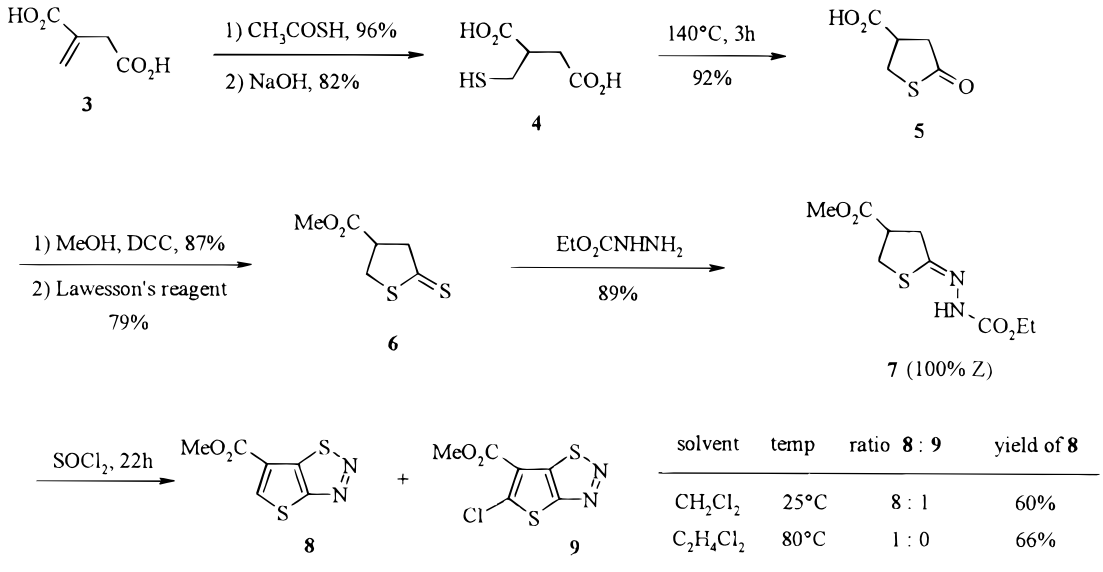

Fig. 2. Synthetic route for thiano[2,3-*d*][1,2,3]thiadiazole-6-carboxylate.

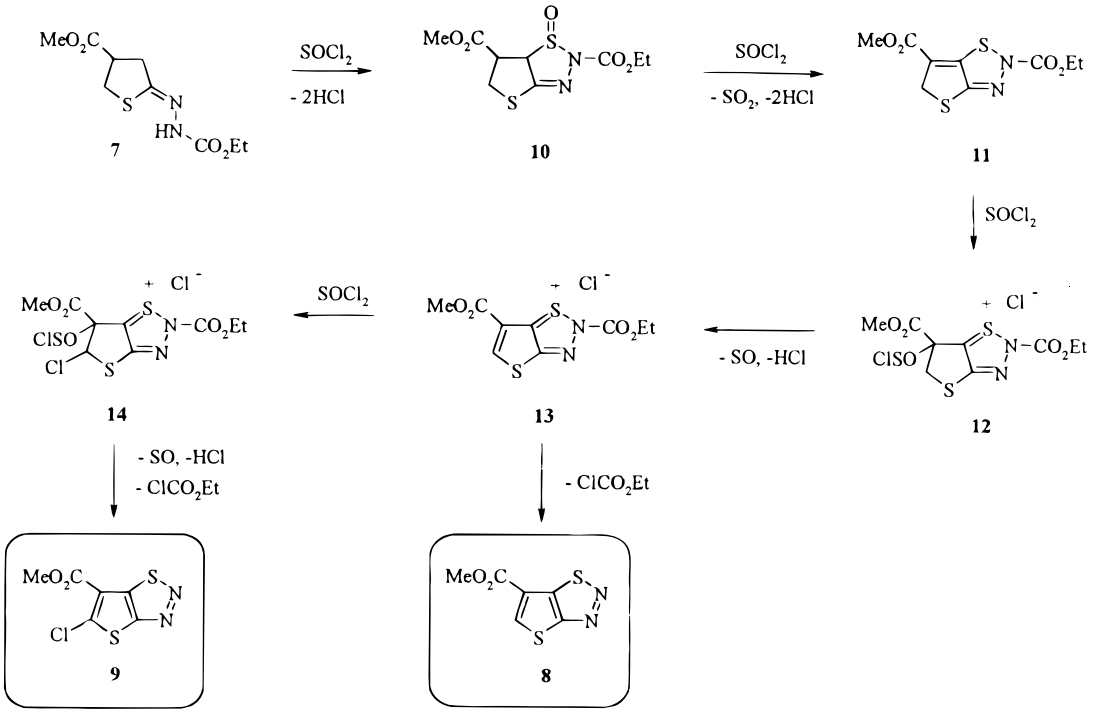

Fig. 3. Mechanisms of formation of thiano[2,3-*d*][1,2,3]thiadiazole-6-carboxylates and their 5-chloro analogue.

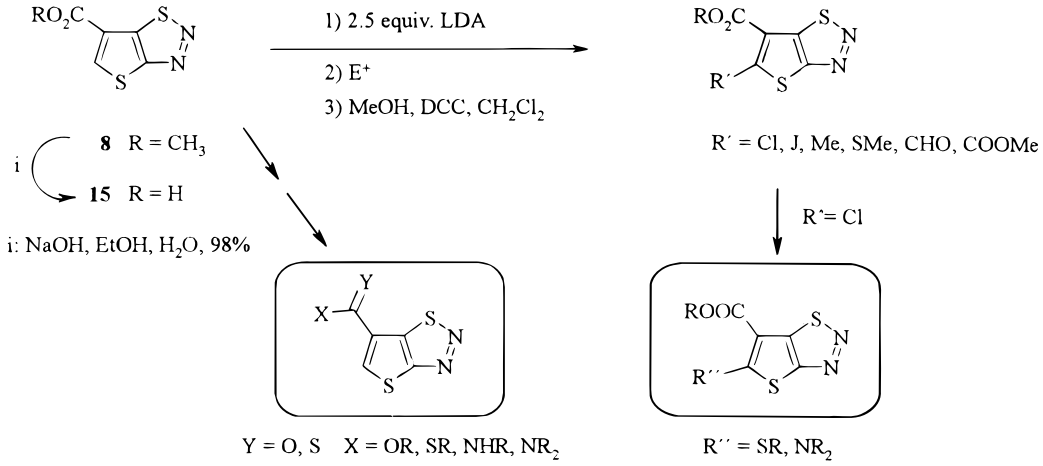

Fig. 4. Synthesis of thiano[2,3-*d*][1,2,3]thiadiazole-6-carboxylate derivatives.

access to this compound. As attempts to introduce the chlorine by electrophilic chlorination failed, this transformation was achieved via *ortho*-metallation[11] of the carboxylic acid **15** with lithium diisopropyl amide and subsequent quenching of the organolithium intermediate with hexachloroethane.[12] In the last step, the chlorinated carboxylic acid **16** was again esterified with dicyclohexyl carbodiimide and methanol. This method was later extended successfully to the introduction of other substituents. For structure–activity studies a large number of derivatives with widely modified carboxylic acid functionalities was synthesised and more 5-substituted analogues were available via nucleophilic displacement of chlorine in compound **9** (Fig. 4).

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Insensitive Acetylcholinesterase Causes Resistance to Organophosphates in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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Abstract: Organophosphates are valuable insecticides used to control *Helicoverpa armigera* on cotton in Australia. Those most commonly used for *Helicoverpa* spp. control are profenofos, parathion-methyl and chlorpyrifos. However, there is an emerging organophosphate-resistance threat in Australian *H. armigera*, which is compounded by cross-resistance between profenofos and parathion-methyl. An insensitive acetylcholinesterase has been identified as the common resistance mechanism. No resistance to chlorpyrifos has been detected and acetylcholinesterase remains fully sensitive to the chlorpyrifos oxon.

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Key words: organophosphate; insensitive acetylcholinesterase; resistance; *Helicoverpa armigera*

Insecticide resistance in the cotton bollworm *Helicoverpa armigera* Hübn. is a continuing threat to the economic production of cotton in Australia.[1–6] Chemical insecticides are currently essential for the control of *H. armigera* on cotton and are likely to remain an important component of control strategies for the foreseeable future. The development of resistance had been delayed by an insecticide resistance management strategy for *H. armigera*, but levels of resistance have gradually increased.[1,6–8] Organophosphates have long been used for *H. armigera* control, and are effective larvicides, but they were not used routinely on cotton since more cost-effective insecticides were available. However, as resistance to pyrethroids, endulsulfan and carbamates has increased, so has the use of alternative chemicals such as the organophosphates, particularly late in the cotton growing season.

While resistance to profenofos was detectable from time to time by bioassay, organophosphate resistance did not give control problems in the field.[4] More recently, however, increasing resistance of *H. armigera* to alternative control chemicals such as endosulfan, pyrethroids and carbamates has resulted in an expanded use of the organophosphates (parathion-methyl, profenfos and chlorpyrifos) on cotton and other crops and, as a consequence, there is an emerging and serious field resistance problem in Australia.[9] This situation has been compounded by bioassay studies which indicate cross-resistance between profenofos and parathion-methyl in *H. armigera*. Selection of field-collected *H. armigera* by profenofos resulted in a high level of resistance in third-instar larvae, to both profenofos (92-fold) and parathion-methyl (52-fold). *H. armigera* larvae remain susceptible to chlorpyrifos.

Our biochemical studies have identified an insensitive acetylcholinesterase (AChE) as the resistance mechanism causing resistance to parathion-methyl, and which presumably causes cross-resistance between that compound and profenofos in Australian *H. armigera*. AChE from the profenofos-resistant *H. armigera* was approximately eight times less sensitive to inhibition by paraoxon-methyl and was also less sensitive to inhibition by profenofos, than AChE from susceptible *H. armigera*. Acetylcholinesterase remains fully sensitive to chlorpyrifos. It is not yet clear how this is associated with insensitive AChE responsible for methomyl and thiodicarb resistance, recently reported in this species.[3]

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